Embryonic PCNA: a missing link?

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PCNA, the processivity factor of the eukaryotic replicative DNA polymerase δ [1], is the functional homolpgue of the β subunit of *Escherichia coli* DNA polymerase III holoenzyme [2,3]. Biochemical studies have shown that the *E. coli* β subunit acts as a sliding clamp on duplex DNA; it has a ring shape that encircles DNA and it binds directly to Pol III, tethering it to DNA to achieve highly processive synthesis [4]. The crystal structure of the β subunit indeed showed it to be a dimeric closed ring with a central cavity large enough to encircle DNA [5]. Each monomer is composed of three globular domains, each with the same chain-folding pattern and very nearly the same three-dimensional structure, so the dimer has a six-fold symmetry (Fig. 1). The PCNA amino-acid sequence is only two-thirds the length of that of the β subunit, so PCNA was thought to have only two domains per monomer and to trimerize to form a six-domain ring [5]. Biochemical data have been obtained that demonstrate PCNA to be a sliding clamp (like the β subunit), and the crystal structure of *Saccharomyces cerevisiae* PCNA shows it is a six-domain ring, arranged as a trimer as predicted [6]. The structural similarities are striking, as the chain folds of the globular domains are the same within the β subunit and PCNA.

To determine at which point in evolution the clamp changed from a dimer to a trimer, we searched the databases for β-subunit and PCNA sequences. The six completely sequenced prokaryotic β genes (*dnaN*) encode similar-sized proteins (365–378 residues), and 12 PCNA genes encode proteins approximately two-thirds this size (256–268 residues). A possible missing link between dimer and trimer was found, in a form of PCNA observed during embryogenesis of *Daucus carota*, the carrot. Two distinct PCNA genes were isolated from *D. carota* [7], one encoding a 264-residue polypeptide (29.2 kD), and the other a 365-amino-acid polypeptide (40.1 kD). These PCNAs, like their *E. coli* and human counterparts, are acidic proteins. The short PCNA is 88 % identical to the amino terminal two-thirds of the long form and shares 65 % identity with human PCNA. The longer PCNA is expressed (along with the short form) during carrot somatic embryogenesis, when cells proliferate rapidly. This may not be the only example of a long PCNA form in embryogenesis: Leibovici *et al.* [8] identified a large PCNA transcript during oogenesis of *Xenopus laevis* and detected a 43 kD protein that cross-reacts with anti-PCNA antibodies.

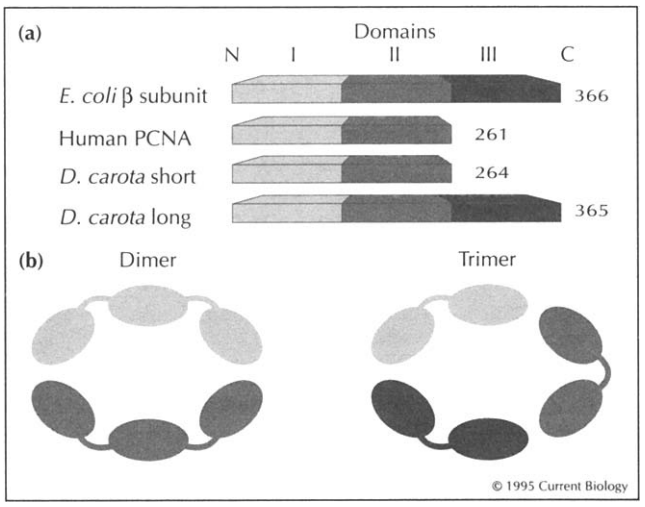

Fig. 1. Dimeric and trimeric sliding clamps. **(a)** Comparison of the *E. coli* β subunit, human PCNA, and short and long forms of carrot PCNA. **(b)** Schematic representation of the three-dimensional structures of the dimeric and trimeric sliding clamps. The long form of carrot PCNA is proposed to have a dimeric structure.

The embryonic long form of PCNA is only one amino acid shorter than the *E. coli* β subunit, suggesting that carrot embryonic PCNA may form a dimeric ring, like the β subunit (Fig. 1). Perhaps a dimeric PCNA provides the rapid chromosomal replication needed during early embryogenesis. This may be an alternative or additional molecular strategy to that used by *Drosophila*, in which an increase in the number of replication origins is used to promote rapid replication in early stages of development [9]. Use of a dimer ring, like that of prokaryotes, during embryogenesis in a eukaryote seems to provide a molecular case of 'ontology recapitulating phylogeny', a phrase inspired by the morphological similarities of embryos at different developmental stages to the morphology of the organisms from which their parents evolved. Perhaps the long embryonic form of PCNA is rooted in the dimeric clamp of a distant prokaryotic ancestor.

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